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(54) Title: A T CELL RECEPTOR SEQUENCE SPECIFICALLY ASSOCIATED WITH AN IMMUNE DISEASE

(57) Abstract

The invention relates to a polypeptide comprising an amino acid sequence contained in the variable region of a Vβ chain of a T cell receptor associated with an immune disease, characterized in that said amino acid sequence is the sequence according to SEQ ID NO: 2 or a fragment or functional equivalent thereof. Also part of the invention are the nucleic acid molecule coding for said polypeptide, and antibodies with and against said amino acid sequence. Furthermore methods to detect the immune disease or the predisposition for the immune disease and vaccines and pharmaceutical formulations for the treatment of the immune disease belong to the invention.

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A T CELL RECEPTOR SEQUENCE SPECIFICALLY ASSOCIATED WITH AN IMMUNE DISEASE.

The invention relates to a polypeptide containing a variable region of a ß chain of a T cell receptor associated with an immune disease, to a T cell receptor, immunogenic compounds and recombinant antibodies comprising this polypeptide, antibodies against this polypeptide, nucleic acids coding for the polypeptide, methods for detection of an immune disease, vaccines and pharmaceutical formulations for prevention and treatment of immune diseases.

Specific T cells are often involved in immune diseases. In the case of autoimmune diseases these T cells are directed to autoantigens and they initiate an immune response to these antigens. However, in many autoimmune diseases the nature of the autoantigen is unknown.

An effective and specific therapy would be directed to the autoreactive T cells without affecting the normally active immune cells. But the autoreactive T cells are only discernible from other T cells by their ability to react with the autoantigen.

If the antigen is unknown, however, it is virtually impossible to select the specific T cell from a pool of T lymphocytes.

The autoreactive T cell is characterized by it's receptors that mediate antigen recognition. These receptors are transmembrane proteins extending from the surface of the T cells. The receptor recognizes a complex of an antigen and MHC (major histocompatibility complex) molecule on other cells.

T cell receptors contain two subunits, designated TCRα and TCRß or TCRγ and TCRδ. These subunits, like iimmunoglobulin L and H chains, consist of variable and constant regions. Hypervariable sequences (CDRs), enabling the specificity of the T cell, are present in the so-called V-regions of TCRα and TCRß, forming the binding site for the epitope. The most variable CDR is CDR3.

In order to detect T cells related to an immune disease it has been tried to search for a unique T cell receptor sequence. Paliard et al. (Science 253, 325-329, 1991), Pluschke et al. (Eur. J. Immunol. 21, 2749-2754, 1991) and Howell et al. (Proc. Natl. Acad. Sci. USA 88, 10921-10925, 1991) have isolated T cells from patients with autoimmune diseases but they found no common T cell receptor CDR3 sequence among different patients.

We have now found a specific amino acid sequence contained in the CDR3 of the ß chain of a T cell receptor which is present in several patients suffering from the immune disease rheumatoid arthritis. According to this finding it is anticipated that this T cell receptor is directed against a common epitope for rheumatoid arthritis.

The invention thus comprises a polypeptide comprising an amino acid sequence contained in the variable region of a ß chain of a T cell receptor associated with an immune disease, characterized in that said amino acid sequence is the sequence according to SEQ ID NO:2 or a fragment thereof or a functional equivalent thereof.

The term "polypeptide" refers to a molecular chain of amino acids, does not refer to a specific length of the product and if required can be modified in vivo or in vitro, for example by glycosylation, amidation, carboxylation or phosphorylation; thus inter alia

peptides, oligopeptides and proteins are included within the definition of polypeptide.

The oligopeptides according to the invention have an amino acid sequence of at least 10 amino acids. More preferably the oligopeptides according to the invention have an amino acid sequence of 10-35, in particular 10-25 amino acids. Highly preferred are oligopeptides with an amino acid sequence of 10-15 amino acids.

As used herein "fragment" refers to any sequence of amino acids that is part of the amino acid sequence depicted in SEQ ID NO:2, which still displays the functional characteristics of the amino acid sequence of SEQ ID NO:2. These functional characteristics of the sequence of SEQ ID NO:2 are the ability to react with the epitope to which the sequence of SEQ ID NO:2 is directed or the ability to raise antibodies in an immune reaction, which antibodies also will bind to the sequence of SEQ ID NO:2. The amino acid sequence LFTGGS (SEQ ID NO:12)for example is such a fragment which displays the same functional characteristics as the sequence of SEQ ID NO:2. Polypeptides comprising an amino acid sequence according to SEQ ID NO:12 are therefor within the scope of the invention.

As used herein, "functional equivalent" means variations of the described sequence still maintaining functional characteristics of the sequence of SEQ ID NO:2.

The variations that can occur in a sequence may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions that are expected not to essentially alter biological and immunological activities, have been described. Amino acid replacements between related amino acids or replacements which have occurred frequently in

evolution are, inter alia Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val (see Dayhof, M.D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3). Based on this information Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science 227, 1435-1441, 1985) and determining the functional similarity between homologous polypeptides.

The polypeptide is in substantially pure form, which means that it is free of other biochemical moieties with which it is normally associated in nature. Such substantially pure polypeptides, for instance, can be synthesized, or produced recombinantly by means known to those skilled in the art. In addition, whole T cell receptors can be treated enzymatically to produce polypeptides according to the invention.

Specifically the amino acid sequence depicted in SEQ ID NO:2 is associated with rheumatoid arthritis (RA).

In rheumatoid arthritis, a disease which affects millions of people, the nature of the autoantigen is completely unknown. The T cells associated with the disease are mainly located in the synovia of the joints which are affected. However, it has been uncertain whether all T cells present in the synovium recognize one or more antigens and are thereby actively contributing to the disease, or whether a substantial number of cells are only passively trafficking through the joint without involvement in the disease. The amino acid sequence depicted in SEQ ID NO:2 of the present invention enables the detection of RA-related T cells and of the autoantigen(s) with which these T cells are reactive. Detection of the presence of the T cell receptor specific amino acid sequence depicted in SEQ ID NO:2 in patients or in a

sample obtainable from patients enables an improved recognition of the autoimmune disease related T cell. Such a detection can be performed by diagnostic methods using immunochemical reagents derived from the polypeptide of the invention.

The polypeptide of the invention not only enables the development of a diagnostic method indicating the presence of T cells having this receptor sequence, but the identified sequence can also serve as a target for specifically directed therapeutic compounds.

Furthermore the polypeptide of the invention can be used to identify the nature of the autoantigen responsible for the immune disease.

The present invention provides a method of immunotherapy for T cell receptor mediated pathologies, including autoimmune diseases, which avoids many of the problems associated with previously suggested methods of treatment. By vaccinating with the polypeptide of the invention the host's immune system is mobilized to suppress the autoaggressive T cells or, in case of passive immunization, antibodies directed to said T cells are administered.

The polypeptide according to the invention can be linked to an immunogenic carrier to further increase its immunogenicity, resulting in an immunogenic compound. Suitable immunogenic carriers are, for instance, keyhole limpet haemocyanin (KLH), human or bovine serum albumine (HSA, BSA) or ovalbumine.

Instead of the polypeptide according to the invention antibodies or fragments of antibodies which are specifically reactive with the amino acid sequence having SEQ ID NO:2 can be employed in immunotherapy

for T cell receptor mediated pathologies in rheumatoid arthritis.

These antibodies can be produced by performing an active immunization of a suitable mammal with the polypeptide according to the invention. This immunization with the polypeptide of the invention will give rise to the formation of antibodies directed to the variable region of this polypeptide.

Said antibodies, fragments of antibodies, or functional derivatives of these antibodies or fragments form also part of the invention. A fragment of an antibody is defined as that part of an antibody which is able to react with and is specific for an antigen. These fragments can be obtained by enzymatic reactions from the original antibody but they also can be made by chemical synthesis or via recombinant DNA methods. A functional derivative of an antibody is a compound which is also reactive with the variable region of the polypeptide according to the invention. Such compounds can be formed, for instance, by incorporating the amino acids constituting the complementary determining regions (CDR's) of an antibody in another amino acid sequence.

When polyclonal antibodies are desired, techniques for producing and processing polyclonal sera are known in the art (e.g. Mayer and Walter, eds, Immunochemical Methods in Cell and Molecular Biology, Academic Press, London, 1987). In short, a selected mammal, e.g. a rabbit is given (multiple) injections with one of the above-mentioned immunogenic compounds, e.g. corresponding to about 20 μg to about 80 μg of polypeptide per immunization. Immunization is carried out with an acceptable adjuvant, generally in equal volumes of immunogen and adjuvant. Acceptable adjuvants include Freund's complete, Freund's incomplete, alum-precipitate or water-in-oil emulsions, with a preference for Freund's complete

adjuvant for the initial immunization. For booster immunization Freund's incomplete adjuvant is preferred. The initial immunization consists of the administration of approximately 1 ml emulsion at multiple subcutaneous sites on the backs of the rabbits. Booster immunizations utilizing an equal volume of immunogen are given at about one monthly intervals and are continued until adequate levels of antibodies are present in an individual rabbits serum. Blood is collected and serum isolated by methods known in the art.

Monospecific antibodies to each of the immunogens are affinity purified from polyspecific antisera by a modification of the method of Hall et al. (Nature 311, 379-387 1984), prepared by immunizing rabbits as described above with the purified polypeptides. Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogeneous binding characteristics for the relevant antigen. Homogeneous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, in this particular case binding to the amino acid sequence SEQ ID NO:2.

Monoclonal antibody reactive against the amino acid sequence depicted in SEQ ID NO:2 can be prepared by immunizing inbred mice, preferably Balb/c with the appropriate protein by techniques known in the art (Kohler and Milstein, Nature 256; 495-497, 1975). Hybridoma cells are subsequently selected by growth in hypoxanthine, thymidine and aminopterin in an appropriate cell culture medium such as Dulbecco's modified Eagle's medium (DMEM). Antibody producing hybridomas are cloned, preferably using the limiting dilution technique as described in Current Protocols in Immunology, Eds: J.A Coligan et al., Greene Publ. Ass. and Wriley-Interscience, 1992. Individual colonies are cultivated in an appropriate culture

medium. Antibody producing cells are identified by screening with the appropriate immunogen. Immunogen positive hybridoma cells are maintained by techniques known in the art. Specific anti-monoclonal antibodies are produced by cultivating the hybridomas in vitro or preparing ascites fluid in mice following hybridoma injection by procedures known in the art.

Antibodies can be isolated from the culture of the immortalized lymphocytes.

When used therapeutically there is, however, a problem with antibodies of animal origin. Upon repeated administration they will give rise to an anti-antibody response in man. It is therefore preferred to use humanized antibodies or small parts of antibodies which will not lead to an immune response. Methods for humanizing antibodies, such as CDR-grafting, are known (Jones et al., Nature 321, 522-525, 1986). Methods for producing fragments of antibodies which are still specific for the antigen of the original antibody are also known (Udaka et al., Molec. Immunol. 27, 25-35; 1990). Another possibility to avoid antigenic response to antibodies reactive with polypeptides according to the invention is the use of human antibodies or fragments or derivatives thereof.

Human antibodies can be produced by in vitro stimulation of isolated B-lymphocytes, or they can be isolated from (immortalized) B-lymphocytes which have been harvested from a human being immunized with at least one polypeptide according to the invention.

Another aspect of the invention are molecules which can interact specifically with the epitope for which the T cell receptor of the invention is specific. These can be obtained by synthesizing recombinant antibodies through genetic engineering. In this case one of the complementary determining regions of such a recombinant

antibody will be formed by the amino acid sequence according to SEQ ID NO:2. This can be accomplished by methods known in the art, preferably by CDR grafting (Jones et al., Nature 321, 522-525, 1986). In this way it is possible to prepare antibodies comprising the amino acid sequence having SEQ ID NO:2 according to the invention in one of their CDR's.

Furthermore, it lies within the skill of the art to produce anti-idiotype antibodies which recognize the 'antigen' binding site of the antibody and therefore are an "internal image" of the 'antigen'. In the case of anti-idiotypic antibodies against antibodies against the T cell receptor the 'antigen' is formed by the receptor. In case of anti-idiotypic antibodies against recombinant antibodies as discussed above the 'antigen' constitutes the antigen binding to these recombinant antibodies, i.e. the antigen for which the T cell receptor is specific.

These anti-idiotype antibodies will be also very useful for treatment and diagnostic purposes.

T cell receptors comprising the amino acid sequence of SEQ ID NO:2 are also within the scope of the invention. Preferably the sequence depicted in SEQ ID NO:2 or a fragment thereof or its functional derivative is included in a ß chain of a T cell receptor. This receptor can be used to search for the antigen(s) which specifically bind to this receptor. Methods to use this kind of receptors in such a way have been described by Hickling et al. (Eur. J. Immunol. 22, 1983-1987, 1992).

Furthermore, these receptors are particularly useful for the detection of the corresponding epitope of the receptor.

An epitope is defined as the specific surface of an antigen molecule, which is delineated by the area of

interaction with a T cell receptor or antibody. Knowledge of this epitope, of the antigen on which it can be found and of the presence of the epitope in the various organs and tissues will give more insight in the onset, spread and development of the immune disease.

A T cell receptor comprising the amino acid sequence according to the invention will be reactive with the epitope-MHC complex. Localization of such a T cell receptor can be traced if said receptor has been labeled. Labeling can be done according to methods known in the art and labels that can be used are, for instance, enzymes, fluorescent compounds, dyes and radioactive compounds.

If such a reaction is performed in vitro the antigen-T cell receptor complex can be isolated. It is possible then to characterize the antigen and to determine the epitope that binds to the polypeptide of the invention.

Furthermore the T cell receptor can be used for diagnosis and therapy. For this purpose preferably the T cell receptor is solubilized in aqueous solutions.

Also covered by the invention is a nucleic acid sequence coding for an amino acid sequence of the polypeptide of the invention.

Preferably the nucleic acid sequence coding for the polypeptide comprises the sequence shown in SEQ ID NO:1 or a sequence which hybridizes to said sequence or its complementary strand under stringent conditions. Said hybridizing sequences still encode for a polypeptide which is functional equivalent to the polypeptide of the invention. "Stringent conditions" in hybridizing experiments are formed by a combination of a relatively high temperature and a relatively high concentration of salts in the solution. Next to the temperature and salt concentration the stringency is also determined by the number of nucleotides that are hybridized, by the A-T

content and the number of mismatches of said hybridization reaction.

Furthermore the degeneracy of the genetic code also means that the nucleic acid sequence of SEQ ID NO:1 codes for a certain amino acid sequence and the same amino acid sequence can be derived using a different nucleic acid sequence in which the codons for certain amino acids are different to those of the sequence of SEQ ID NO:2. The various nucleic acid sequences coding for the amino acid sequence according to SEQ ID NO:2 also fall within the scope of the invention.

One of the aspects of the invention is to provide an immunochemical reagent which can be used in diagnostic test kits for detecting the presence of, or the predisposition for an immune disease.

Such an immunochemical reagent can comprise compounds such as a polypeptide according to the invention, a T cell receptor comprising the amino acid sequence according to SEQ ID NO:2 or an antibody comprising said amino acid sequence and antibodies reactive with said amino acid sequence having SEQ ID NO:2.

The term "immunochemical reagent" signifies that the compounds mentioned above are bonded to a suitable support or are provided with a labeling substance.

The supports which can be used are, for example, the inner wall of a microtest well, a tube or capillary, a membrane, filter, test strip or the surface of a particle such as, for example, a latex particle, an erythrocyte, a dye sol, a metal sol or metal compound as sol particle.

Labeling substances which can be used are, inter alia, a radioactive isotope, a fluorescent compound, an enzyme, a dye sol, metal sol or metal compound as sol particle.

In a method for the detection of, or predisposition for, an immune disease, an immunochemical reagent according to the invention is used, which reagent is brought into contact with the test fluid, and the presence of immune complexes, formed between the immunochemical reagent and its counterpart in the test fluid, is detected and from this the presence of the immune disease or a predisposition for this disease can be derived. Depending on the nature of the immunochemical reagent according the invention the nature of the counterpart which is being detected in the test fluid varies. The following list shows which elements can be detected with the specified immunochemical reagents:

Immunochemical reagent comprising:	counterpart:
polypeptide according to the invention:	epitope/antigen
T cell receptor comprising amino acid sequence of SEQ ID NO:2:	epitope/antigen
antibody comprising amino acid sequence of SEQ ID NO:2:	epitope/antigen
antibody reactive with amino acid sequence of SEQ ID NO:2:	T cell

The immunochemical reaction which must take place when using these detection methods is preferably a sandwich reaction, an agglutination reaction, a competition reaction or an inhibition reaction.

A test kit according to the invention must contain, as an essential constituent, an immunochemical reagent such as described above. For carrying out a sandwich reaction, the test can consist of the - unlabelled - immunochemical reagent bonded to a solid support, for example the inner wall of a microtest well, it being

possible to use a labeled immunochemical reagent for the detection.

For carrying out a competition reaction, the test kit can consist of the immunochemical reagent bonded to a solid support, a labeled antibody directed against this reagent then being used to compete with compounds in the test fluid.

In an agglutination reaction an immunochemical reagent bonded to particles or sols must be brought into direct contact with the test fluid in which the counterpart to the immunochemical reagents which is to be detected is present.

A testkit can also comprise one or more nucleic acid sequences according to the invention. In this case the nucleic acid of the test kit will hybridize with complementary strands, if present, and the hybridized product can be detected by agents capable of discrimination of hybridized nucleic acids.

Preferably a testkit comprising a nucleic acid will be an amplification kit in which the hybridized strands are amplified in order to facilitate easy detection.

Also a preferred embodiment of the above described testkit is a kit in which one of the nucleic acid sequences which hybridizes with the DNA sequences of the invention is labeled so that it facilitates detection of the hybridized strands.

Next to the detection of, or predisposition for an immune disease another use of a diagnostic kit is the detection of circulating antibodies directed to vaccinated compounds to monitor a therapeutic effect of administration of vaccination with the polypeptide according to the invention.

Treatment of immune diseases can be established by the polypeptide of the invention, by nucleic acids according to the invention or by antibodies according to the invention. These compounds can be the basis of a pharmaceutical formulation.

Very suitable for the treatment of immune disease are vaccines comprising the polypeptide comprising the amino acid sequence of SEQ ID NO:2, the T cell receptor comprising the amino acid sequence of SEQ ID NO:2, an immunogenic compound comprising the amino acid sequence of SEQ ID NO:2 or an antibody specifically reactive with the amino acid sequence of SEQ ID NO:2.

For therapeutic purposes, the polypeptide according to the invention can be formulated with pharmaceutical acceptable carriers for administration.

Pharmaceutical acceptable carriers are well known to those skilled in the art and include, for example, sterile salin, lactose, sucrose, calcium phosphate, gelatin, dextrin, agar, pectin, peanut oil, olive oil, sesame oil, and water.

In addition the pharmaceutical composition according to the invention, may comprise one or more adjuvants. Suitable adjuvants include, amongst others, aluminum hydroxide, aluminum phosphate, amphigen, tocophenols, monophosphenyl lipid A, muramyl dipeptide and saponins such as Quill a. The amount of adjuvant depends on the nature of the adjuvant itself.

Furthermore the pharmaceutical composition according to the invention may comprise one or more stabilizers such as , for example, carbohydrates, including sorbitol, mannitol, starch, sucrosedextrin, and glucose, proteins such as albumin or casein, and buffers like alkaline phosphates.

To increase the immunogeneity of the polypeptide in a vaccine according to the invention, said polypeptide can be coupled to an immunogenic carrier. Conventional

immunogenic carriers include tetanus toxoid, Keyhole lympet haemocyanin (KLH), Human and Bovine serum albumine (HSA, BSA). This is in particular useful when oligopeptides comprising the amino acid sequence according to SEQ ID NO:2 are used.

Suitable administration routes are intramuscular, subcutaneous, intravenous or intraperitoneal injections, oral administration or nasal sprays.

The amount of active ingredient administered will depend on the route of administration, the time and frequency of administration, the age of the person treated as well as general health conditions and diet.

In general, a dosage of 0.01 to 1000 µg, preferably 0.05 to 500 µg, more preferably 0.1 to 100 µg of polypeptide per kg of host can be used.

For vaccination a pharmaceutical composition comprising a polypeptide according to the invention is administered to a patient suffering from an immune disease. The administration will give rise to an immune response. Antibodies or T cells will be stimulated which recognize this polypeptide as well as the corresponding amino acid sequence of the variable region on the β chain of the T cell receptor. These antibodies or T cells will specifically bind to this receptor thereby preventing that this T cell will initiate or maintain an immunological reaction. In this way only the specific T cell population carrying the amino acid sequence according to SEQ ID NO:2 of the invention will be influenced.

For passive immunization instead of the polypeptide the antibodies reactive against the amino acid sequence of SEQ ID NO:2 or fragments or functional equivalents thereof are directly administered. The antibodies specifically reactive with the amino acid sequence of SEQ ID NO:2 are very suitable for use in a vaccine in

case of passive vaccination. For this purpose the antibodies can be formulated in essentially the same way as the polypeptides according to the invention.

Thus, antibodies have to be raised against the polypeptides according to the invention. This is achieved through an active immunization scheme of a suitable mammal as explained earlier. It will be clear to one skilled in the art that the polypeptide of the invention can be bound to a carrier molecule to enhance the immunogenicity.

As already discussed above passive immunization can be used for the treatment of immune disease. In this case complete antibodies will be produced by an immunized animal. Also fragments of these antibodies, or functional derivatives of these antibodies or their fragments are useful.

A preferred embodiment is a conjugate of such an antibody, fragment, or functional derivative, with a cytotoxic substance. The antibody is then used as targeting moiety which will bind to the 'antigen' for which it is specific. In this case the 'antigen' will be for instance the T cell receptor comprising the amino acid sequence depicted in SEQ ID NO:2 according to the invention. The antibody will bind to it's 'antigen' and preferably be internalized. In any case the toxic compound is brought in or in the neighbourhood of the cell carrying the 'antigen'. In this way the toxin can act locally and thus specifically.

It is also possible to use the nucleic acid sequences of the invention for therapeutic purposes. Anti-sense strands to the strand coding for the polypeptide of the invention will block translation of the DNA and thus prohibit the formation of the specific T cell receptor. More specifically a conjugate of an anti-sense strand and a toxin can be administered. After hybridization to the target sequence the toxin can exert its toxic

effects thereby destroying the DNA or other cell components. With these hybridization therapies only T cells expressing the specific target sequence are addressed because only in these T cells the specific genetic rearrangement takes place, which results in the expression of the T cell receptor comprising the amino acid sequence according to SEQ ID NO:2. Such a therapy can prevent stimulation of pathological mechanisms without employing cell destruction mechanisms.

Conjugation of label and/or toxic compounds with a polypeptide, a nucleic acid, an antibody, fragment or analogue thereof or a receptor according to the invention can be performed by normal chemical methods or by recombinant DNA techniques.

Toxic compounds which can be coupled to the compounds according to the invention are for instance mitomycin, Pseudomonas exotoxin, diphteria toxin or derivatives, adriamycin, anthracycline derivatives, ricin, trichothecenes, calicheamycins, dynemycin, camptothecin, actinomycin D, ansamycins, amanitin, bleomycins, alpha emitting isotopes and all other compounds which are specifically cytotoxic.

Preferably the pharmaceutical compound is mixed with one or more pharmaceutical acceptable carriers, e.g. as described in the standard reference Chase et al., Remington's Pharmaceutical Sciences. By means of pharmaceutically suitable liquids the compound can also be applied as an injection preparation in the form of a solution, suspension, emulsion, or as a spray, e.g. a nasal spray.

EXAMPLE 1

Isolation of T cells

Synovia infiltrated with T cells were obtained from 11 patients suffering from rheumatoid arthritis (RA). Patients fulfilled the ARA (American Rheumatology Association) criteria for definitive clinical RA and a clinical score of the joints from which the synovia were used was determined from X-ray photographs.
Synovia were minced and incubated in complete medium (an equal mixture of Dulbecco's modiefied Eagle Medium (DMEM, Gibco 074-2100) and Nutrient mixture F12 (HAM's F12, Gibco 074-1100), supplemented with 2500 mg/l sodium bicarbonate, 55 mg/l sodium pyruvate, 2.3 mg/l ß-mercaptoethanol, 1.22 mg/l ethanolamine, 260 mg/l L-glutamine, $4.5 \cdot 10^{-4}$ mg/l sodium selenite, 62.5 mg/l sodium penicillin, 62.5 mg/l streptomycin sulphate and 10% human pooled AB serum to which was added 200 U/ml collagenase and 0.1 mg/ml DNase I (obtained from Sigma).
After an incubation of 90 min. the cells were filtered over a 200 µm nylon filter and mononuclear cells were isolated by Ficoll Hypaque density centrifugation. The cells were incubated in a tissue culture flask in complete medium for 16 hours and non-adherent cells were collected and stimulated. Mononuclear cells from peripheral blood (PBL) for control experiments were isolated by Ficoll Hypaque density centrifugation, collected and stimulated.
For stimulation with Interleukine-2 (IL-2), $2 \cdot 10^5$ mononuclear cells per well were seeded in 96 wells roundbottom plates (obtained from Nunc) in 200 µl complete medium supplemented with 10 U/ml IL-2. For further expansion of T cell lines, $2 \cdot 10^4$ T cells and $2 \cdot 10^5$ allogeneic irradiated (2500 rad) PBL were seeded in roundbottom microwells in 200 µl complete medium

supplemented with 10 U/ml IL-2 and 1.25 µg/ml PHA (Phytohaemagglutinin, Wellcome) or 0.1 µg/ml anti-CD3 (OKT3, obtainable from ATCC).

In Table 1 HLA class DR typing of the patients is given. It can be seen that with exception of patient 04 all patients carry a DR haplotype associated with rheumatoid arthritis. T cell lines that could be used for V-gene analysis were derived from 9 patients. From 5 patients (04, 06/07, 08, 10 and 14) IL-2 expanded lines both from synovia and PBL were obtained that allowed for a comparative analysis of V-gene usage in both compartments.

Table 1. HLA-DR typing of patients.

Patient	DR type
01	0405-1301
03	0102-0701
04	1501-11
05	0401
06/07	0401-11
08	0401-0407
10	0405-1401
11	0401-0701
12	0405-0701
14	0101-0401
15	0405-1501

The T cell lines derived from the synovia were characterized by FACS (Fluorescence Activated Cell Sorter) analysis for the expression of TCR-Âß chains and gamma-_. The results are depicted in Table 2.

Markers for B cells (CD19), NK cells (CD 16) and monocytes (CD 14) were absent in all lines, except SCRO.11.CD3, which contained 36% CD 16^+ cells. The percentage of $CD4^+$ and $CD8^+$, double positive and double negative T cells was determined. All lines expressed MHC class II antigens and were of the $CD45RA^-$ subtype. All lines with the exception of SCRO.04 and SCRO.08 consisted mainly of ÂB T cells. CD4/CD8 ratios varied and shifts in the ratio could not be consistently related with the different stimulation protocols.

Table 2A

synovial cells	PHA STIMULATION							
	CD3	CD4	CD8	$CD4^+$ $CD8^+$	$CD4^-$ $CD8^-$	CD4/ CD8	TCR αβ	TCR δ
SCRO.01	98	52	40	<2	<2	1.3	98	<2
SCRO.03	98	>60	12	<2	<2	>5	98	<2
SCRO.04	99	41	54	<2	<2	0.76	ND	ND
SCRO.05	>90	10	90	<2	<2	0.11	ND	ND
SCRO.06	95	21	83	<2	<2	0.25	ND	ND
SCRO.07	100	17	81	<2	<2	0.21	97	2
SCRO.08	90	>80	<2	<2	<2	>40	>80	<2
SCRO.10	100	36	>62	<2	<2	<0.58	ND	ND
SCRO.11	95	28	62	<2	<2	0.45	95	<2
SCRO.12	98	35	60	<2	<2	0.5	98	<2
SCRO.14	99	40	33	<2	<2	1.2	99	<2
SCRO.15	not available							

Table 2B

synovial cells	anti-CD3 STIMULATION							
	CD3	CD4	CD8	$CD4^+$ $CD8^+$	$CD4^-$ $CD8^-$	CD4/ CD8	TCR αβ	TCR δ
SCRO.01	95	76	20	<2	<2	3.8	95	<2
SCRO.03	100	95	4	<2	<2	24	99	<2
SCRO.04	100	35	37	ND	25	0.95	70	25
SCRO.05	100	13	87	ND	<2	0.15	100	<2
SCRO.06	100	66	82	47	<2	0.80	94	<2
SCRO.07	100	47	50	ND	<2	1.14	100	<2
SCRO.08	96	48	10	ND	38	4.8	55	41
SCRO.10	93	55	37	<2	10	1.5	93	2
SCRO.11	43	11	38	ND	ND	0.29	44	<2
SCRO.12	100	42	54	4	12	0.78	85	5
SCRO.14	92	58	35	<2	8	1.7	92	<2
SCRO.15	100	<2	97	ND	<2	<0.02	100	<2

Table 2C

synovial cells	IL-2 STIMULATION							
	CD3	CD4	CD8	$CD4^-$ $CD8^+$	$CD4^+$ $CD8^-$	CD4/ CD8	TCR αβ	TCR δ
SCRO.01	98	20	72	<2	<2	0.28	98	1
SCRO.03	100	80	11	<2	<2	7.3	97	3
SCRO.04	not available							
SCRO.05	not available							
SCRO.06	88	21	>69	<2	<2	0.30	87	<2
SCRO.07	100	65	9	<2	<2	7.2	100	<2
SCRO.08	99	71	28	<2	<2	2.6	100	<2
SCRO.10	95	87	8	<2	<2	12	>80	<2
SCRO.11	100	80	19	<2	<2	4.2	100	<2
SCRO.12	100	57	31	<2	<2	1.8	95	7
SCRO.14	85	24	>63	<2	<2	0.38	91	<2
SCRO.15	100	6	91	<2	<2	0.07	100	<2

EXAMPLE 2

Amplification of V-gene families

$1 \cdot 10^6$ T cells were lysed in 200 µl RNAzol (Cinna/Bioteck) and 20 µl chloroform was added. The mixture was incubated on ice for 15 minutes and centrifuged. The RNA in the upper layer was precipitated with an equal volume isopropanol and the precipitate was washed once with 70% ethanol. cDNA was synthesized using Avian Myeloblastoma Virus (AMV) reverse transcriptase and random hexanucleotides (Amersham cDNA synthesis system plus) according to the manufacturers instructions. In Table 3 a list is presented of the primers used for the amplification reactions of the VÂ and Vß gene family from the cDNA preparations according to the method of Choi et al. (Y. Choi et al., Proc. Natl. Acad. Sci. USA 86, 8941-8945, 1989; Kabat E.A., Wu T.T., Reid-Miller M, Perry H.M. and Gottesman K.S., in: Sequences of proteins of immunological intrest. 4th ed. Public Health Service, NIH, Washington DC, 1987) In each reaction a control amplification using TCR constant region primers was done. To the amplification reactions $1 \cdot 10^5$ cpm of ^{32}P-labeled 3′ primers and 2.5 units of Taq (Thermus aquaticus) polymerase (Perkin-Elmer Cetus) were added. The temperature profile used for amplifications was 1 minute 94°C, 1 minute 55°C, 1 minute 72°C for 30 cycles. The reaction products were analyzed on a 2% agarose gel. The gel was dried and autoradiographed (Fig. 1). The expression level was estimated from the intensity of the amplification products in relation to the intensity of the amplification product of the TCR constant region that was used as an internal standard. The results of the analysis of all T cell lines tested is given in Fig. 2.

A mean of 11 to 12 VÂ families of the 18 analyzed and 15 VB families of the 20 analyzed were expressed in the synovial T cell lines.

Table 3. T-cell receptor primers

Primer	5'--> 3' sequence	family members
5'Cα	GAACCCTGACCCTGCCGTGTACC	
3'Cα	ATCATAAATTCGGGTAGGATCC	
5'Cβ	CCGAGGTCGCTGTGTTTGAGCCAT	
3'Cβ	CTCTTGACCATGGCCATC	
Vα1	TTGCCCTGAGAGATGCCAGAG	
Vα2	GTGTTCCCAGAGGGAGCCATTGCC	1.1, 1.2, 1.3
Vα3	GGTGAACAGTCAACAGGGAGA	2.1, 2.2
Vα4	ACAAGCATTACTGTACTCCTA	3.1
Vα5	GGCCCTGAACATTCAGGA	4.1
Vα6	GTCACTTTCTAGCCTGCTGA	5.1
Vα7	AGGAGCCATTGTCCAGATAAA	6.1
Vα8	GGAGAGAATGTGGAGCAGCATC	7.1, 7.2
Vα9	ATCTCAGTGCTTGTGATAATA	8.1, 8.2
Vα10	ACCCAGCTGGTGGAGCAGAGCCCT	9.1
Vα11	AGAAAGCAAGGACCAAGTGTT	10.1
Vα12	CAGAAGGTAACTCAAGCGCAGACT	11.1
Vα13	GCTTATGAGAACACTGCGT	12.1
Vα14	GCAGCTTCCCTTCCAGCAAT	13.1
Vα15	AGAACCTGACTGCCCAGGAA	14.1
Vα16	CATCTCCATGGACTCATATGA	15.1
Vα17	GACTATACTAACAGCATGT	16.1
Vα18	TGTCAGGCAATGACAAGG	17.1
Cα3'	AATAGGCAGACAGACTTGTCACTGGA	18.1
Vβ1	GCACAACAGTTCCCTGACTTGCAC	
Vβ2	TCATCAACCATGCAAGCCTGACCT	1.1-2
Vβ3	GTCTCTAGAGAGAAGAAGGAGCGC	2.1-3
Vβ4	ACATATGAGAGTGGATTTGTCATT	3.1-2
Vβ5.1	ATACTTCAGTGAGACACAGAGAAAC	4.1-3
Vβ5.2-3	TTCCCTAACTATAGCTCTGAGCTG	5.1
Vβ6.1-3	AGGCCTGAGGGATCCGTCTC	5.2-3
Vβ7	CCTGAATGCCCCAACAGCTCTC	6.1-3
Vβ8	ATTTACTTTAACAACAACGTTCCG	7.1-2
Vβ9	CCTAAATCTCCAGACAAAGCTCAC	8.1-4
Vβ10	CTCCAAAAACTCATCCTGTACCTT	9.1
Vβ11	TCAACAGTCTCCAGAATAAGGACG	10.1-2
Vβ12	AAAGGAGAAGTCTCAGAT	11.1-2
Vβ13.1	CAAGGAGAAGTCCCCAAT	12.1-2
Vβ13.2	GGTGAGGGTACAACTGGC	13.1
Vβ14	GTCTCTCGAAAAGAGAAGAGGAAT	13.2
Vβ15	AGTGTCTCTCGACAGGCACAGGCT	14.1
Vβ16	AAAGAGTCTAAACAGGATGAGTCC	15.1
Vβ17	CAGATAGTAAATGACTTTCAG	16.1
Vβ18	GATGAGTCAGGAATGCCAAAGGAA	17.1
Vβ19	CAATGCCCCAAGAACGCACCCTGC	18.1
Vβ20	AGCTCTGAGGTGCCCCAGAATCTC	19.1
3'Cβ	TTCTGATGGCTCAAACAC	20.1

For 5 patients the IL-2 stimulated T cells of synovium and PBL could be compared (Fig. 3). Differences are observed in the expression patterns of synovial T cells and PBL derived T cells in individual patients. The expression of various VÂ families is markedly increased in the synovial lines, but no single family is consistently found in all patients. In the VB analysis the data presented in Fig. 3 suggest that the largest differences are found in the VB9, 13.1 and 14 gene families.

Sequence analysis

For sequence analysis amplifications were done on cDNA of selected T cell lines using a VB14 family specific primer with a 5' EcoRI linker (5'-gggaattcGTCTCTCGAAAAGAGAAGAGGAAT-3') and a CB primer with a SalI linker (5'-ggggtcgacCCTTTTGGGTGTGGGAGATC-3'). The amplification profile used was 1 min 94°C, 1 min 55°C and 1 min 72°C for 40 cycli. After amplification, the reaction products were cut with EcoRI and SalI and electrophoresed on a 1.7% low melting point agarose gel. The bands were isolated and ligated into pBluescriptSK+ (obtained from Stratagene). E. coli DH5ÂF' cells (obtained from Stratagene) were transformed and minipreparations were made. Purified DNA was used for double strand DNA sequence reactions with T7 DNA polymerase (Pharmacia) using a CB specific primer (5'-TTCTGATGGCTCAAACAC-3').

The results of the sequencing experiments are shown in Table 4. In 9 out of 9 clones derived from patient 08 an identical rearrangement was found containing the amino acid sequence -L-F-T-G-G-S-A-G-A-N-. Sequencing of 12 clones from patient 14 resulted in 12 identical rearrangements, again containing the amino acid

sequence -L-F-T-G-G-S-A-G-A-N-. The results were confirmed in an independent experiment with clones derived from patient 14, where again the same sequence was found in 4 out of 5 clones. The sequences flanking -L-F-T-G-G-S-A-G-A-N- are also found identical but not unique for the polypeptide of the invention because the leading Vß sequence is specific for Vß 14, which was meant to be amplified, and because the trailing sequence is coded on the J part of the receptor sequence, which is less variable than the D part, which codes for the polypeptide of the invention.

Table 4. Sequence data of parts of the Vß sequence of the T cell receptor.

Cell line	Ratio	Vß	CDR3	Cβ
SCRO.IL2 08	9/9	YFCASS	LFTGGSAGANVLTFGAGSRLTVL	EDL
SCRO.IL2 14	12/12	YFCASS	LFTGGSAGANVLTFGAGSRLTVL	EDL
SCRO.IL2 14	4/5	YFCASS	LFTGGSAGANVLTFGAGSRLTVL	EDL
	1/5	YFCAT	KSGRGGYEQYFGPGTRLTVT	EDL

EXAMPLE 3

Preparation of antibodies against the N-acetylated peptide Ac-LFTGGSAGAN (SEQ ID NO:2)

Five Balb/c mice were immunized with the N-acetylated peptide Ac-LFTGGSAGAN (SEQ ID NO:2) conjugated to the immunocarrier KLH (Keyhole Limpet hemocyanin). The peptide-KLH conjugate was dissolved in 40 mM phosphate, 0.9% NaCl, pH 7.4 to a concentration of 1 mg/ml. An amount of peptide-KLH conjugate corresponding to 20 µg peptide was mixed with 20 µl complete (1st immunization) or incomplete (booster) Freunds adjuvant. In each case the total volume was brought to 100 µl by addition of PBS. and the preparations were injected intraperitoneal at 0 and 6 weeks.or at 0. 3, 6, 9 and 12 weeks.

The mice were bled at 6 respectively 12 weeks and the titer of these antibodies against the N-acetyleted peptide was determined in an immunoassay. Plates were coated with the N-acetylated peptide Ac-LFTGGSAGAN coupled to BSA (Bovine Serum Albumine). The mice sera were diluted and 100 µl of the diluted sera was added for 1 hour. After several washes 100 µl of a sheep-anti-mouse-antibody labelled with HRP (Horse Raddish Peroxidase) diluted 1000x was added for 1 hour, whereafter again several washes were performed. The binding of the HRP labelled antibody was detected by the enzymatic conversion of TMBS. 100 µl 4N H_2SO_4 was added and the colour development was measured at 450 nm. The mice sera had an average titer of 1:3000, which is a very good titer. Thus a peptide comprising the amino acid sequence according to SEQ ID NO:2 is immunogenic and is able to raise antisera with very good titers.

From the afore-mentioned mice sera we were able to obtain at least five monoclonal antibodies which were highly reactive with the peptide comprising the amino acid sequence according to SEQ ID NO:2. The monoclonal antibodies were obtained according to standard techniques well known in the art (Coligan et al. (eds), Current protocols in Immunology, 1992; Steenbakkers et al., Mol. Biol. Rep. 19:125-134, 1994).

The anti-peptide response was measured using plates coated with the N-acetylated peptided coupled to BSA. 100 µl of the monoclonal antibodies were added together with the free N-aceylated peptide in a concentration range of 0-0.155-0.313-0.626-1.25-2.5-5-10 µg/well in duplo to the plate. After several wash steps sheep-anti-mouse-antibodies conjugated to HRP (1000x diluted) were added to each well, followed by incubation with the substrate TMBS at roomtemperature. 100 µl 4N H_2SO_4 was added and the absorbance of the wells was determined at 450 nm. The free peptide was able to compete with the peptide coated to the plates and at concentrations varying from 200 ng/ml to 5 µg/ml of free peptide the monoclonal antibodies which were bound to the plate was reduced by 50%.

LEGENDS TO THE FIGURES

Fig. 1. Autoradiograph of amplification products of indicated patient derived T cell lines. The synovium derived cell lines were stimulated by IL-2 (SCRO IL-2), PHA + IL-2 (SCRO PHA) or anti-CD3 + IL-2 (SCRO aCD3). PBL are peripheral blood lymphocytes. Indicated are the bands containing amplification products for Cß, VÂ2, CÂ and Vß1 gene families.

Fig. 2a. Expression of TCR VÂ genes as detected by amplification analysis. Indicated are strong, moderate and low expression observed from autoradiographs as shown in fig. 1 for each VÂ gene family (listed from VÂ1 to VÂ18) and for each T cell line. The synovium derived cell lines were stimulated by IL-2 (SCRO IL-2), PHA + IL-2 (SCRO PHA) or anti-CD3 + IL-2 (SCRO aCD3). PBL IL-2 are peripheral blood lymphocytes stimulated by IL-2.
Fig. 2b. Identical scheme for Vß-gene families.

Fig. 3. Rearrangement of data in Figs. 2a and 2b to show differences in V gene expression.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Akzo Nobel N.V.
(B) STREET: Velperweg 76
(C) CITY: Arnhem
(E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): 6824 BM
(G) TELEPHONE: 04120-66379
(H) TELEFAX: 04120-50592
(I) TELEX: 37503 alpha nl

(ii) TITLE OF INVENTION: A T cell receptor sequence specifically associated with an immune disease.

(iii) NUMBER OF SEQUENCES: 12

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: T-lymphocyte

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /product= "CD3 of T cell receptor"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Leu Phe Thr Gly Gly Ser Ala Gly Ala Asn
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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 1               5                  10
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..8
(D) OTHER INFORMATION: /standard_name= "EcoRI linker"

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 9..32
(D) OTHER INFORMATION: /standard_name= "V beta 14 primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /standard_name= "SalI linker"

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 10..29
(D) OTHER INFORMATION: /standard_name= "C beta primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGGGTCGACC CTTTTGGGTG TGGGAGATC 29

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /standard_name= "C beta primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: T-lymphocyte

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /label= Vbeta
/note= "Vbeta region"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 7..29
(D) OTHER INFORMATION: /label= cdr3
/note= "CDR3 region"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 30..32
(D) OTHER INFORMATION: /label= Cbeta
/note= "Cbeta region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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            20                  25                  30
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(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: T lymphocyte

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /label= Vbeta
/note= "Vbeta region"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 6..25
(D) OTHER INFORMATION: /label= CDR3
/note= "CDR3 region"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 26..28
(D) OTHER INFORMATION: /label= Cbeta
/note= "Cbeta region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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1 5 10 15

Gly Pro Gly Thr Arg Leu Thr Val Thr Glu Asp Leu
20 25

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..26
(D) OTHER INFORMATION: /standard_name= "c-alpha 3' primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AATAGGCAGA CAGACTTGTC ACTGGA 26

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /standard_name= "3'c-beta primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..24
(D) OTHER INFORMATION: /standard_name= "5'c-beta primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: T-lymphocyte

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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 1               5
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(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

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Leu Phe Thr Gly Gly Ser
 1               5
```

CLAIMS

1. Polypeptide comprising an amino acid sequence contained in the variable region of a ß chain of a T cell receptor associated with an immune disease, characterized in that said amino acid sequence is the sequence according to SEQ ID NO:2 or a fragment or functional equivalent thereof.

2. Polypeptide according to claim 1, characterized in that the polypeptide is the ß chain of a T cell receptor.

3. T cell receptor comprising the amino acid sequence according to SEQ ID NO:2 or a fragment or functional equivalent thereof.

4. Recombinant antibody or anytibody fragment comprising the amino acid sequence according to SEQ ID NO:2 or a fragment or functional equivalent thereof.

5. Immunogenic compound, characterized in that the polypeptide according to claim 1 is coupled to an immunogenic carrier.

6. Antibody or antibody fragment, characterized in that it is reactive with the amino acid sequence according to SEQ ID NO:2 or a fragment or functional equivalent thereof.

7. A nucleic acid molecule encoding a polypeptide according to claim 1 or 2.

8. A nucleic acid molecule, characterized in that it comprises the nucleotide sequence shown in SEQ ID NO: 1 or a nucleotide sequence which hybridizes to

said sequence or its complementary strand under stringent conditions.

9. Polypeptide according to claim 1 or 2 for use as a medicament.

10. Pharmaceutical composition comprising a polypeptide according to claim 1 or 2 and a pharmaceutical acceptable carrier.

11. T cell receptor according to claim 3 for use as a medicament.

12. Pharmaceutical composition comprising a T cell receptor according to claim 3 and a pharmaceutical acceptable carrier.

13. Antibody according to claim 4 or 6 for use as a medicament.

14. Pharmaceutical composition comprising an antibody according to claim 4 or 5 and a pharmaceutical acceptable carrier.

15. Immunochemical reagent which comprises a compound selected from the group consisting of a polypeptide according to claim 1 or 2, a T cell receptor according to claim 3 and an antibody or antibody fragment according to claim 4 or 6.

16. Method for the detection of, or predisposition for an immune disease, characterized in that an immunochemical reagent according to claim 11 is used, which reagent is brought into contact with a test fluid and that the presence of immune complexes formed with the immunochemical reagent is detected.

17. Method for the detection of, or predisposition for an immune disease, characterized in that a nucleic acid molecule according to claim 9 or 10 is brought into contact with a test fluid and that the presence of hybridization products with said nucleic acid molecule is detected.

18. Vaccine for the prevention or treatment of an immune disease, characterized in that it comprises a compound selected from the group consisting of a polypeptide according to claim 1 or 2, a T cell receptor according to claim 3, an immunogenic compound according to claim 5, and an antibody or antibody fragment according to claim 4 or 6.

Fig. 1

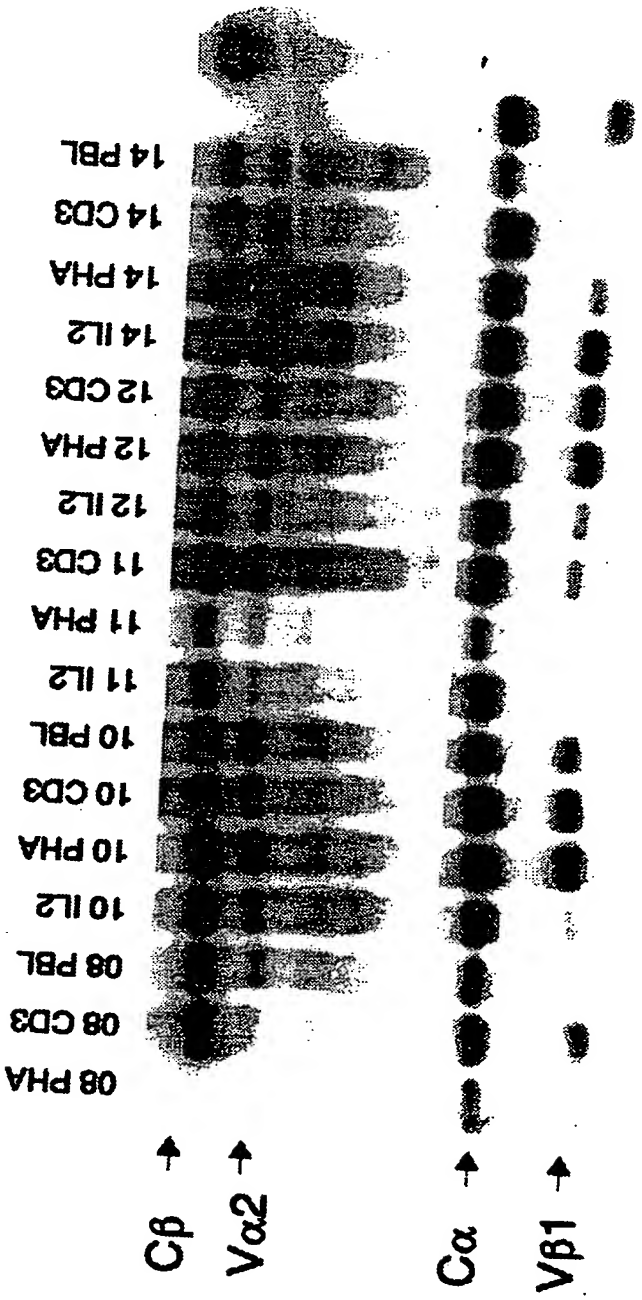


Fig. 2a

EXPRESSION TCR Vα-GENES

SCRO IL-2

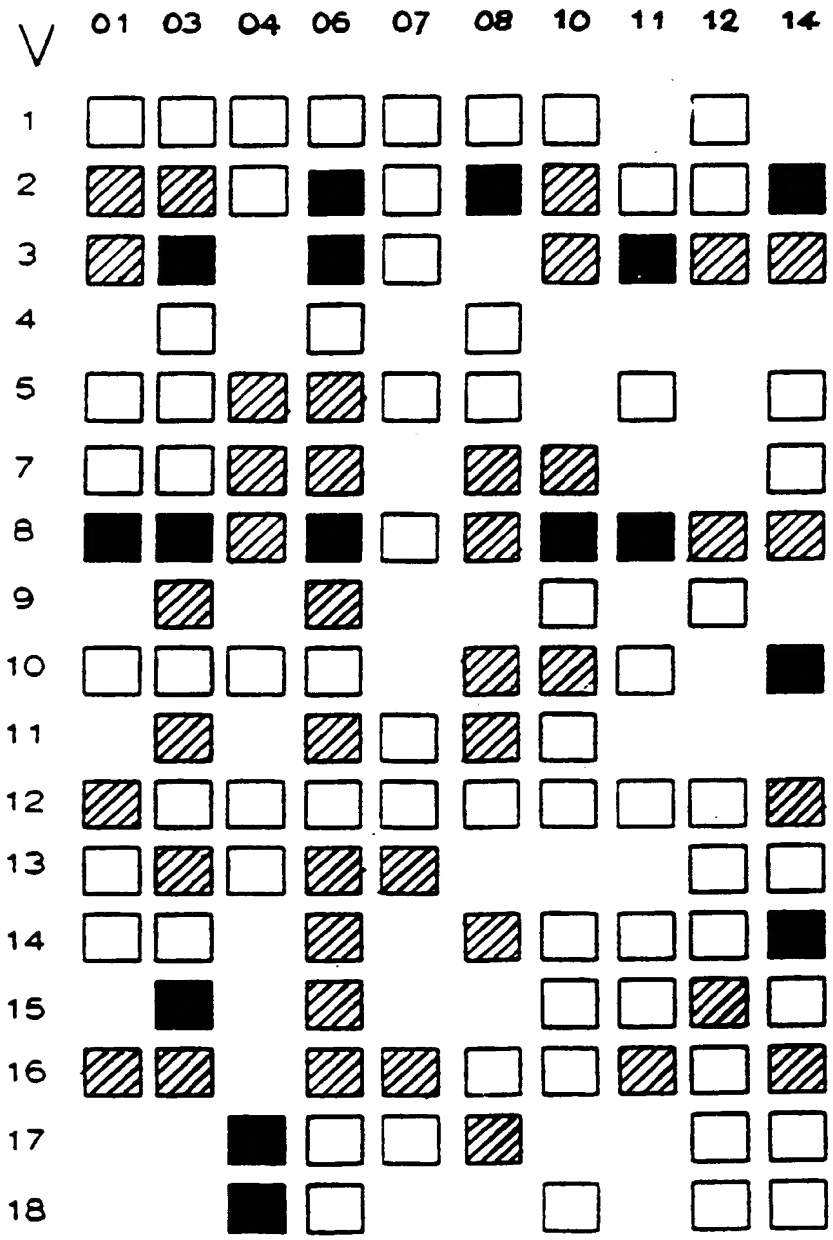


Fig. 2a EXPRESSION TCR Vα-GENES

SCRO PHA

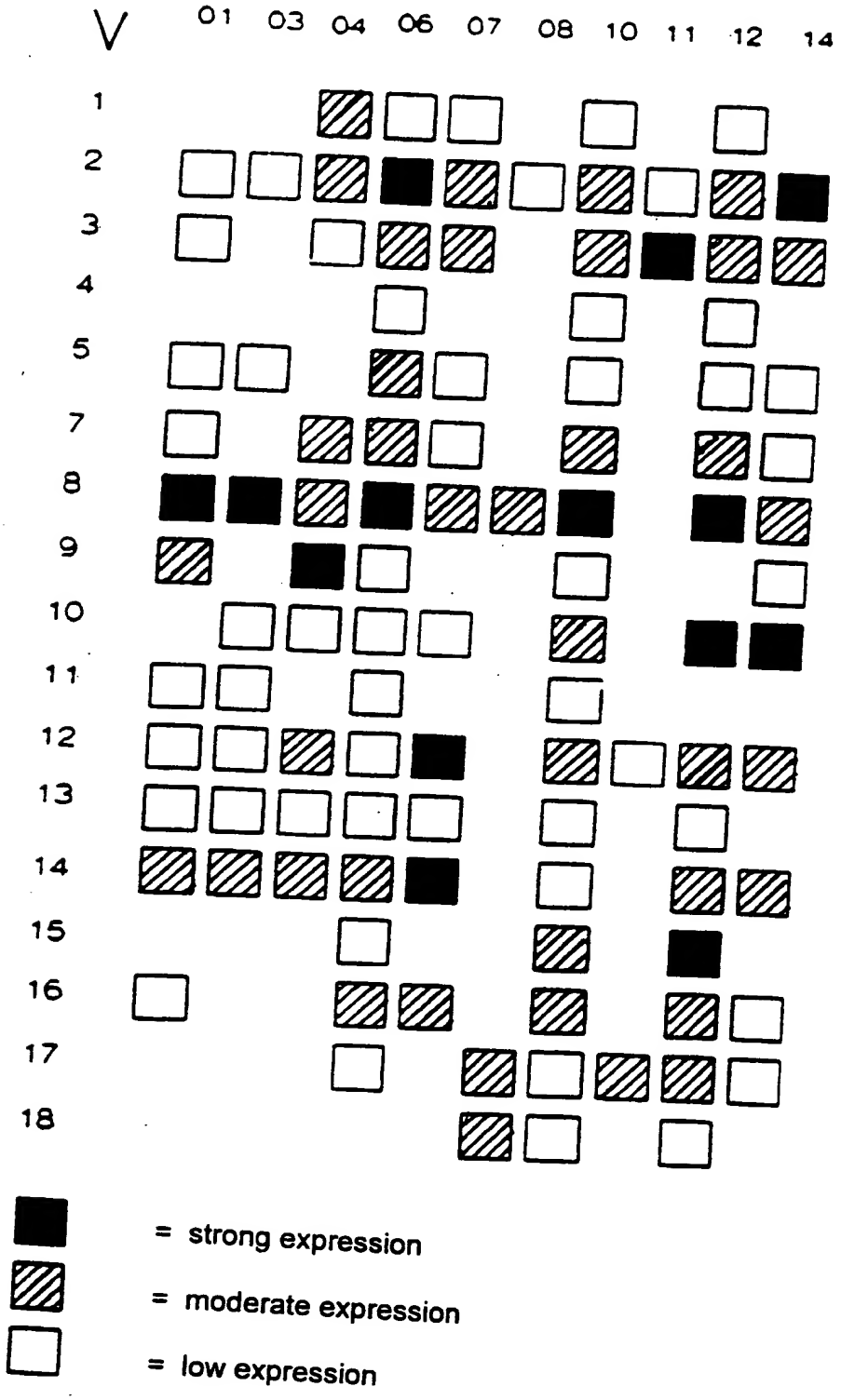


Fig. 2a EXPRESSION TCR Vα-GENES

SCRO aCD3

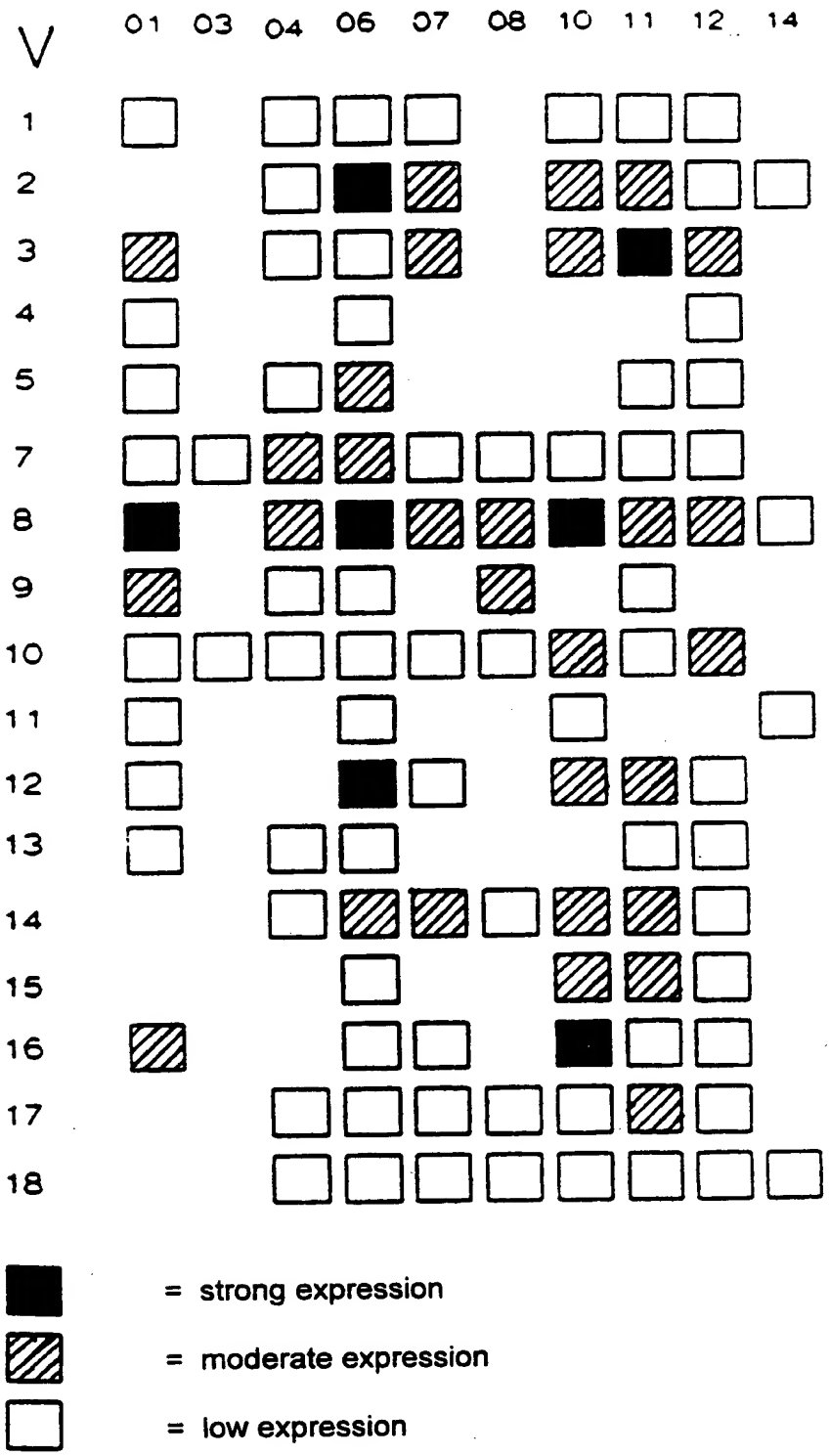
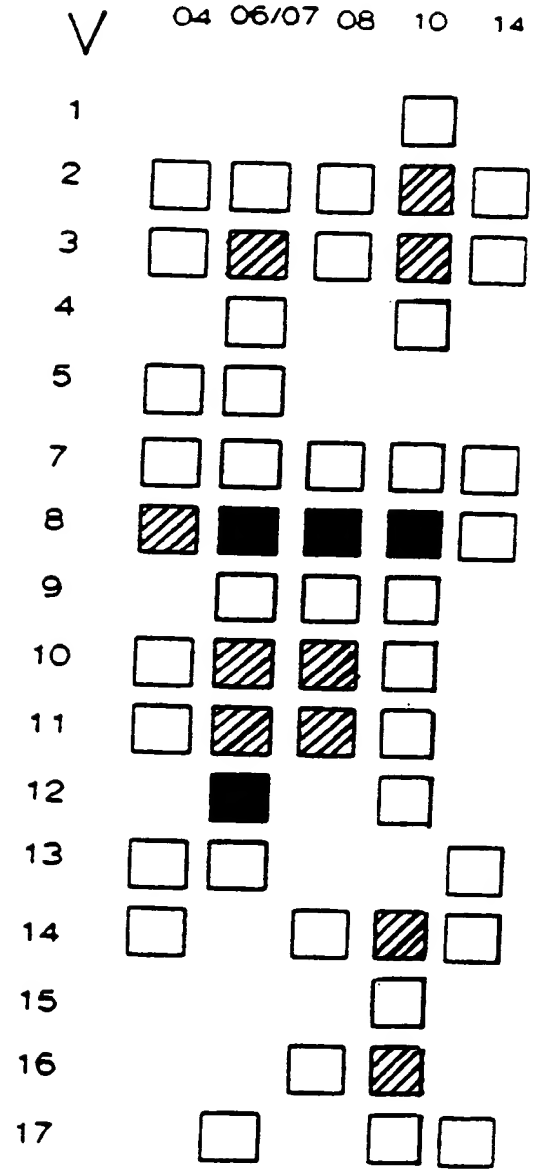


Fig. 2a EXPRESSION TCR Vα-GENES

PBL IL-2



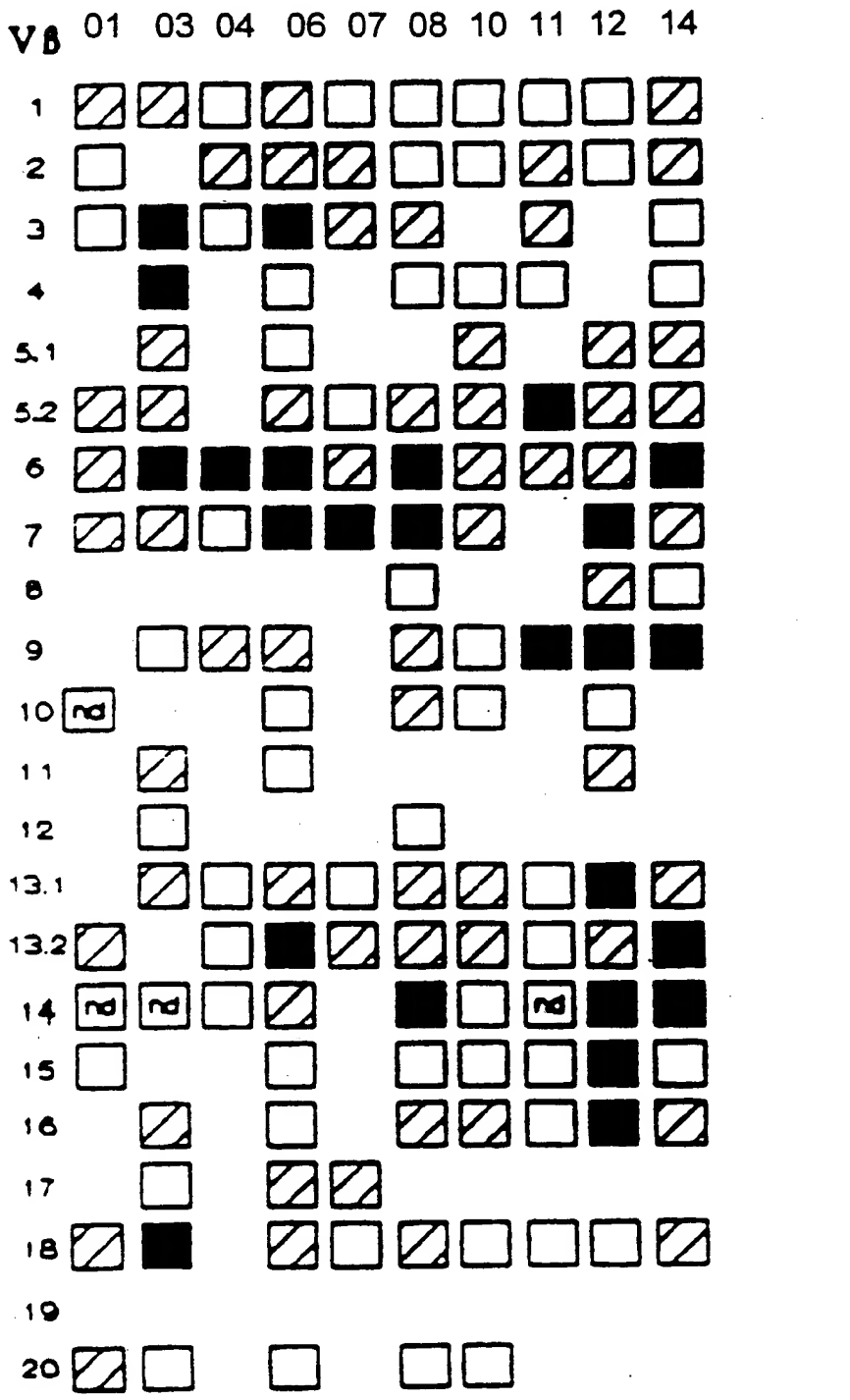
= strong expression

= moderate expression

= low expression

Fig. 2b **EXPRESSION TCR Vβ-GENES**

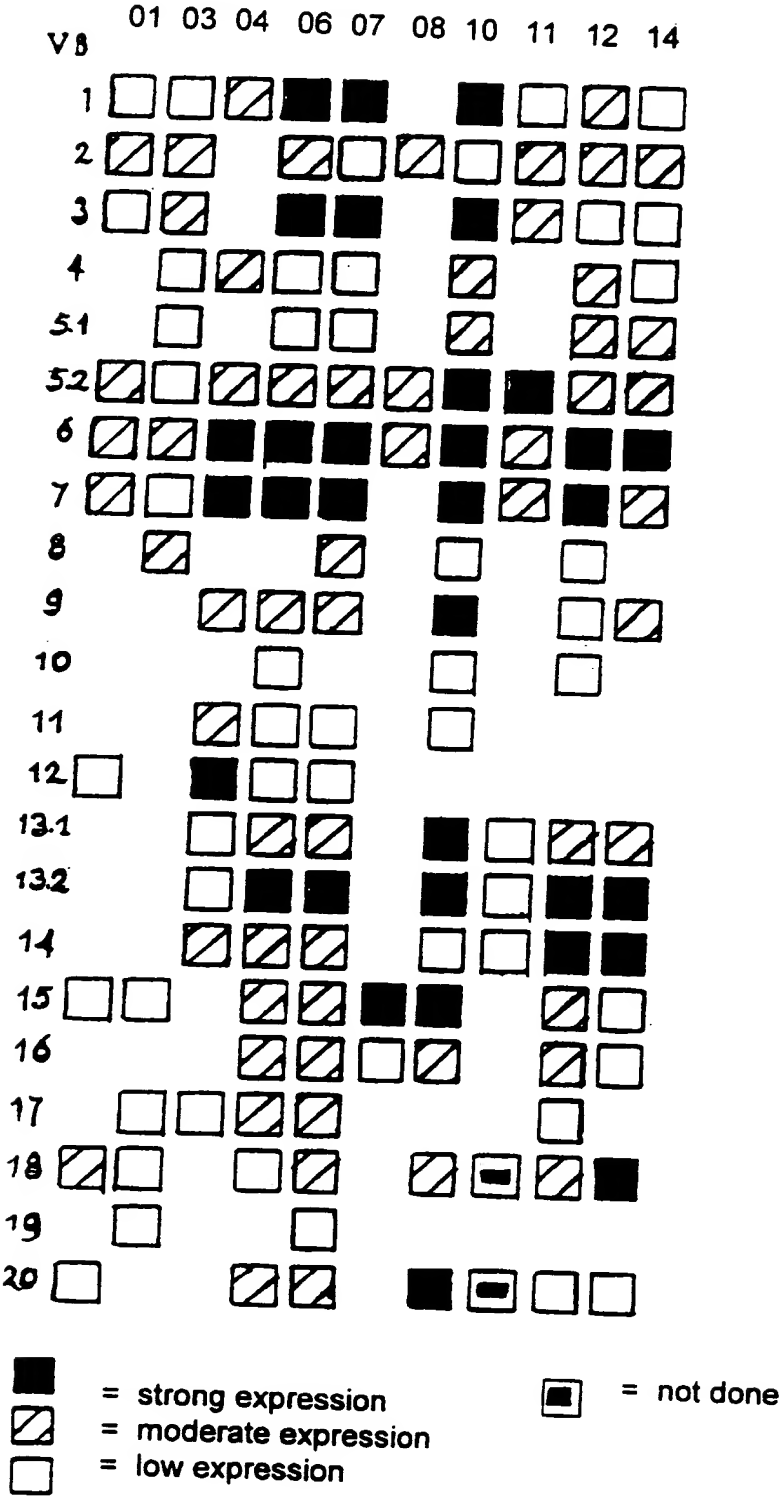
SCRO IL-2



EXPRESSION TCR Vβ-GENES

Fig. 2b

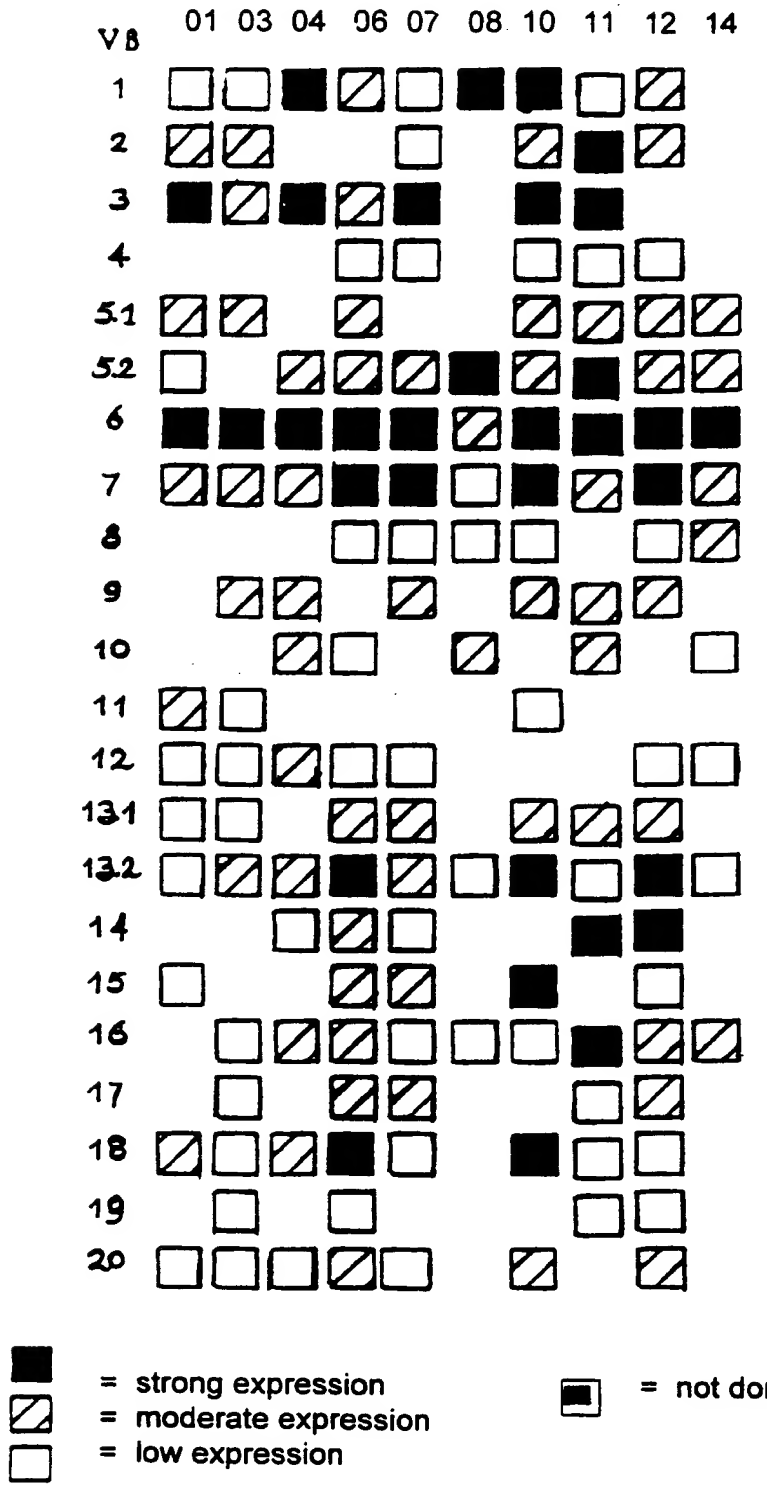
SCRO PHA



EXPRESSION TCR Vβ-GENES

Fig. 2b

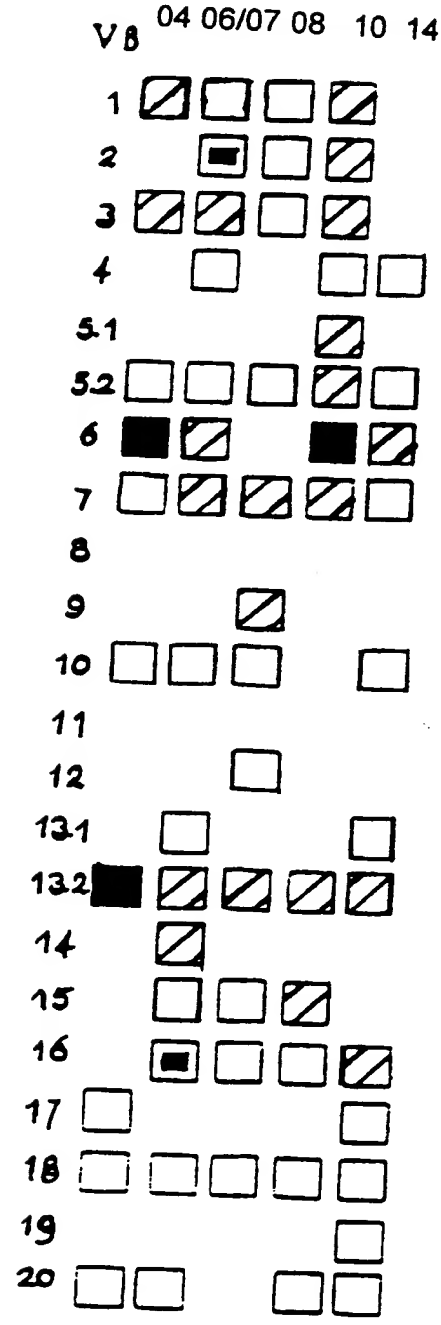
SCRO aCD3



EXPRESSION TCR Vβ-GENES

Fig. 2b

PBL IL-2



= strong expression
= moderate expression
= low expression
= not done

Fig. 3

EXPRESSION TCR Vβ-GENES

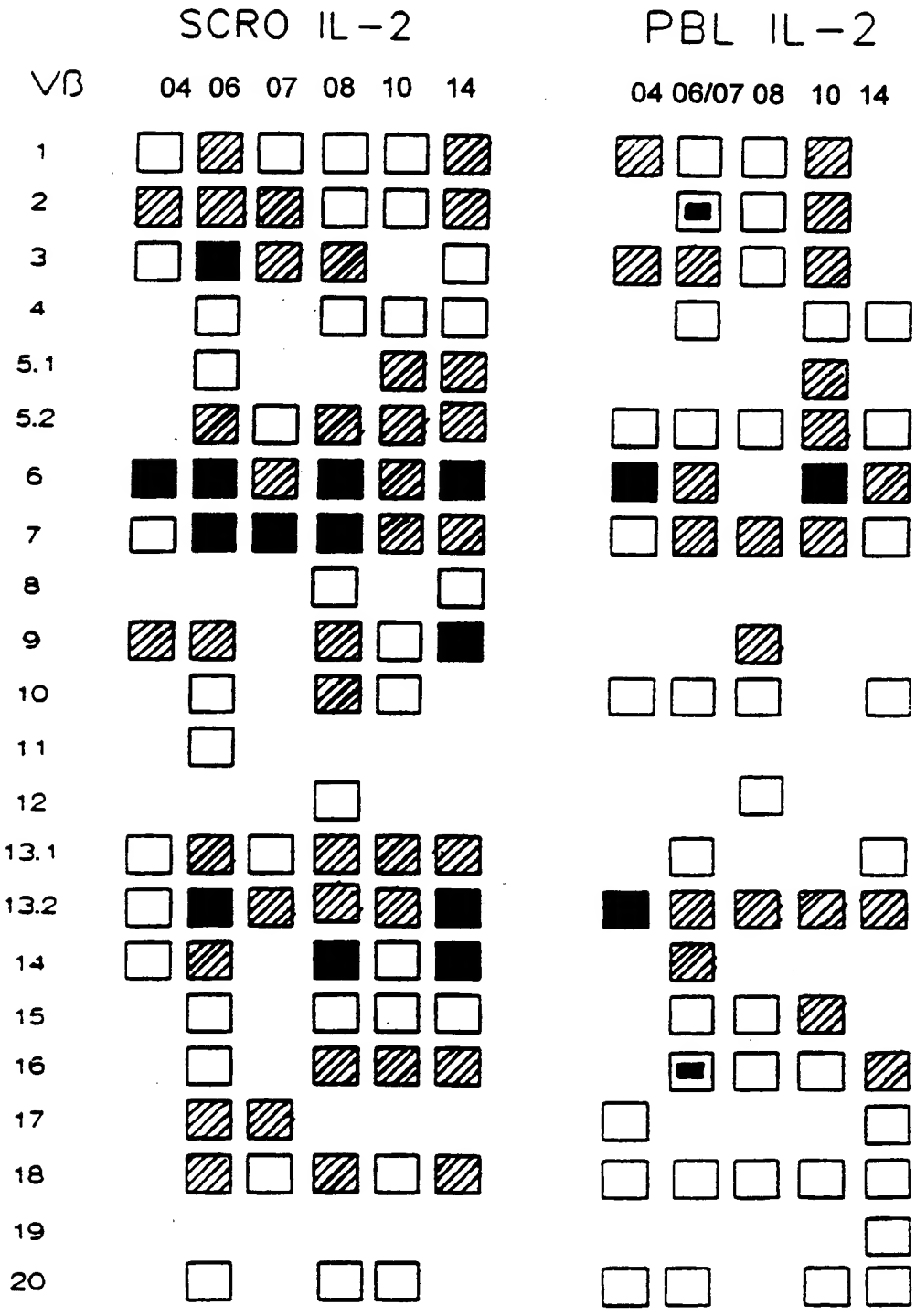
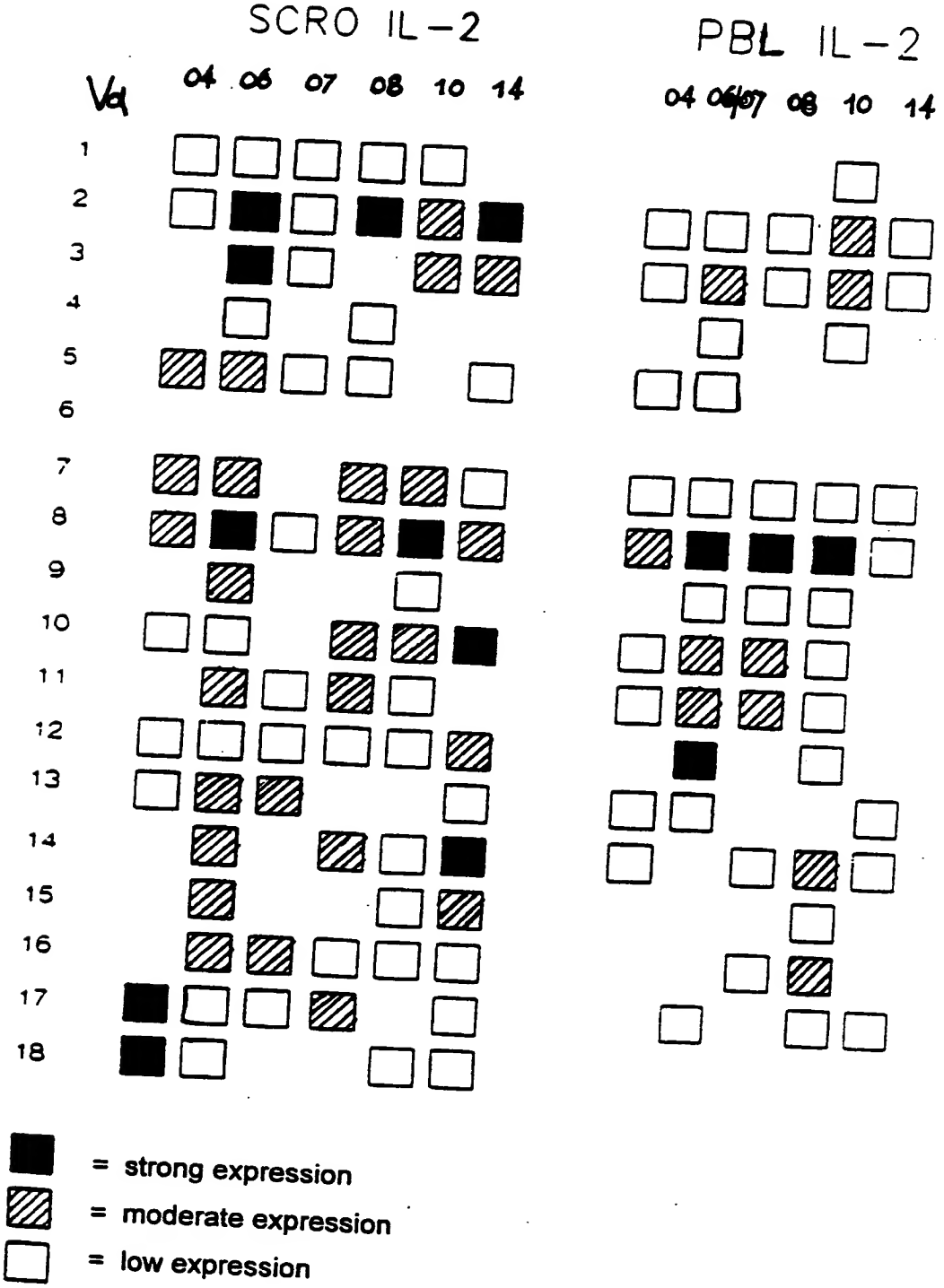


Fig. 3 EXPRESSION TCR Vα -GENES



INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 95/00670

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 7/06, A61K 38/08, A61K 39/395
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, REG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A2, 9212996 (THE IMMUNE RESPONSE CORPORATION), 6 August 1992 (06.08.92) --	1-18
X	WO, A1, 9101133 (VANDENBARK, ARTHUR), 7 February 1991 (07.02.91) --	3

[ ] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 May 1995	06. 06. 95

Name and mailing address of the International Searching Authority:
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INTERNATIONAL SEARCH REPORT

Information on patent family members

01/04/95

International application No.
PCT/EP 95/00670

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		EP-A-	0568623	10/11/93
		JP-T-	6507384	25/08/94
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		JP-T-	5504939	29/07/93